VECTOR LABORATORIES

5′ EndTag™ Nucleic Acid Labeling System

Cat. No. MB-9001

Introduction

The 5′ EndTag™ Nucleic Acid Labeling System facilitates the covalent attachment of a variety of fluorescent dyes, haptens, or affinity tags to the 5’ end of unmodified oligonucleotides or 5’-OH modified DNA or RNA.

The 5′ EndTag™ Kit is ideal for labeling PCR and sequencing primers because a label is attached only at the 5′ end, leaving the 3′ end available for polymerization. The position of the label does not interfere with hybridization or nucleic acid binding and is, therefore, appropriate for binding of capture probes to affinity matrices and for gel shift assays.

Store kit at -20 °C upon receipt.

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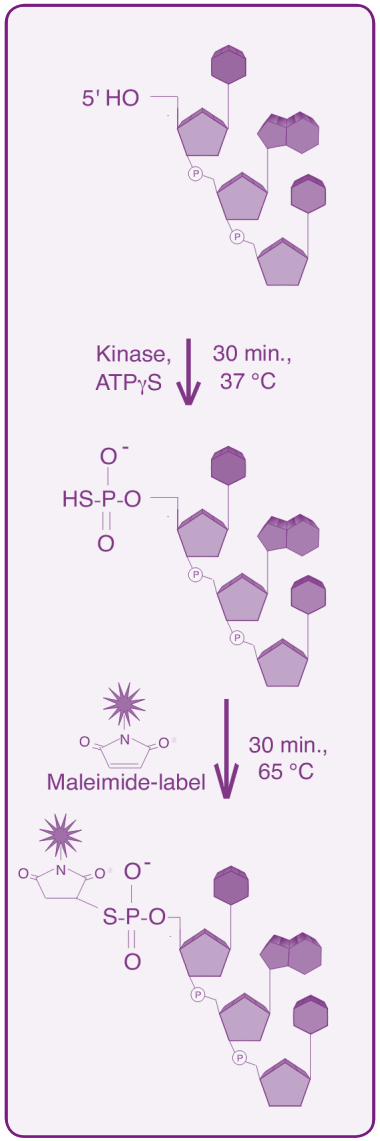
5′ EndTag™ Labeling Reaction

Labeling with the 5′ EndTag™ system requires just a few simple steps (see figure):

1) A thiophosphate is transferred from ATPγS to the 5’ hydroxyl group of a nucleic acid by T4 polynucleotide kinase (PNK).

2) After addition of the thiol functional group, a thiol-reactive label is chemically coupled to the 5’ end of the nucleic acid.



5′ EndTag™ labeling reaction

Kit Components†

- T4 polynucleotide kinase 20 µl
- ATPγS 13 µg
- Universal reaction buffer 30 µl
- Precipitant†† 50 µl
- Alkaline phosphatase††† 10 µl

One kit contains sufficient reagents for 10 reactions of up to 0.6 nmols of 5′ ends per reaction.

† This kit does not include a maleimide label; labels may be selected from the table in note F.

†† Precipitant contains purified glycogen and sodium and magnesium salts.

††† Alkaline phosphatase has been included in the kit to remove the 5′ phosphate group prior to the introduction of the thiophosphate group. Nucleic acids which do not contain a 5′ phosphate, such as unmodified oligonucleotides, require no alkaline phosphatase pretreatment.

Reagent Preparation

- Reconstitute ATPγS in 10 µl of nuclease-free, deionized water.
- Reconstitute maleimide label (not included) as described in note F.

Protocol

If the nucleic acid contains a 5′ phosphate group, begin at Step 1 (see Note A). If the nucleic acid to be labeled has a 5′ hydroxyl group, begin at Step 2.

1. Combine the following in a microcentrifuge tube:

   1 µl universal reaction buffer
   nucleic acid (up to 0.6 nmols of 5′ ends in ≤ 8 µl; see Note B)
   1 µl alkaline phosphatase

   Bring total reaction volume to 10 µl with deionized water. Mix. Incubate for 30 minutes at 37 °C. The entire dephosphorylation reaction mixture can be treated with kinase in Step 2 without purification (see Note C).

2. Combine the following in a microcentrifuge tube (see Notes D and E):

   2 µl universal reaction buffer
   nucleic acid (up to 0.6 nmols of 5′ ends in ≤ 15 µl; see Note B) or entire reaction mixture from Step 1
   1 µl ATPγS
   2 µl T4 polynucleotide kinase

   Bring total reaction volume to 20 µl with nuclease-free, deionized water. Mix. Incubate for 30 minutes at 37 °C.

3. Add 10 µl of thiol-reactive label (see Note F). Mix. Incubate for 30 minutes at 65 °C or 2 hours at room temperature.

4. Add 70 µl of nuclease-free water and 100 µl of buffered phenol (see Note G) and vortex briefly. Remove upper aqueous layer to a clean microcentrifuge tube.

5. To this aqueous fraction add 5 µl of precipitant and 270 µl of 95% ethanol. Mix. Pellet the precipitated nucleic acid by centrifugation at 13,000 x g in a microcentrifuge for 30 minutes. Wash the pellet briefly with 70% ethanol and centrifuge at 13,000 x g for 3 minutes. Dry the pellet and resuspend in TE buffer (10 mM Tris, 1 mM EDTA, pH 8).

6. Applications requiring extremely pure nucleic acids (e.g. in situ hybridization) may require additional purification to remove trace amounts of unincorporated label. For these applications, size exclusion chromatography is recommended.

7. Labeling can be confirmed and semiquantitated by comparing dot blots of the 5′ EndTag™ labeled nucleic acid to those of the labeled control DNA supplied with each maleimide label. Dot 1 ng, 100 pg, 10 pg, and 1 pg of each nucleic acid in 6x SSC (1x = 150 mM NaCl, 15 mM trisodium citrate; pH 7.0) on nitrocellulose or nylon membrane in 1 µl spots. After UV crosslinking, detect by incubating with AP streptavidin (when labeled with biotin maleimide) or with the appropriate AP-labeled antibody and an AP substrate such as BCIP/NBT (Cat. No. SK-5400) or DuoLuX™ Chemiluminescent/Fluorescent Substrate (Cat. No. SK-6605) according to the instructions provided with each product.

Notes:

A. Nucleic acids with a 5′ phosphate (e.g. genomic or restriction-digested DNA, in vitro transcribed RNA or decapped mRNA) may be labeled by either of two methods. In the first, the phosphate is left on and labeling is accomplished by the kinase exchange reaction. This method is simplest (begin at Step 2) but results in lower labeling efficiency. The second method involves enzymatic removal of the 5′ phosphate by alkaline phosphatase (Step 1) followed by labeling by the more efficient kinase forward reaction. Unmodified synthetic oligonucleotides typically contain a 5′ hydroxyl group and, therefore, do not require dephosphorylation before end labeling.

B. The concentration of 5′ ends can be estimated by comparison to the examples below. 0.6 nmols of 5′ ends corresponds to:

- 5 µg of 25 base single-stranded or 25 bp double-stranded nucleic acid
- 10 µg of 50 base single-stranded or 50 bp double-stranded nucleic acid
- 20 µg of 100 base single-stranded or 100 bp double-stranded nucleic acid
- 100 µg of 500 base single stranded or 500 bp double-stranded nucleic acid

Alternatively, use the following formula to calculate nmols of 5′ ends in the nucleic acid to be labeled:

$$\frac{\mathbf{A}}{\mathbf{BxC}} \mathbf{X}\ 1000\ \text{nmols/µmol} = \text{nmols of 5′ ends per µl}$$

where:

A = the concentration of nucleic acid (µg/µl)
B = average molecular weight of nucleotide (333 µg/µmol for DNA; 317 µg/µmol for RNA)
C = total number of bases (for single-stranded DNA or RNA) or the number of base pairs (for double-stranded DNA).

C. The alkaline phosphatase supplied in this kit will not remove the thiophosphate from the nucleic acid. The AP (and PNK) will be heat inactivated in Step 3 and extracted in Step 4.

D. Ammonium ion concentrations greater than 5 mM and NaCl concentrations greater than 50 mM inhibit PNK activity and will decrease labeling efficiency.

E. For nucleic acids containing recessed 5′ ends, labeling efficiency may be improved by incubating the nucleic acid for 5 minutes at 70 °C immediately followed by chilling on ice for 5 minutes just prior to addition to the PNK reaction in Step 2.

F. The following table shows the available thiol-reactive labels and how they should be dissolved for optimal use with the 5′ EndTag™ labeling system:

Thiol-reactive label	***Cat. No.***
Biotin maleimide (dissolve in 312 µl anhydrous DMF)	SP-1501
Fluorescein maleimide (dissolve in 883 µl anhydrous DMSO)	SP-1502
Dinitrophenyl maleimide (dissolve in 120 µl anhydrous DMSO)	SP-1503
Fucose maleimide (dissolve in 100 µl anhydrous DMSO)	SP-1504
Texas Red® maleimide (dissolve in 500 µl anhydrous DMSO)	SP-1505

DMF = dimethyl formamide
DMSO = dimethyl sulfoxide

G. Buffered phenol can be purchased, or prepared as described in many molecular biology protocol manuals (e.g. Sambrook J, Fritsch EF, and Maniatis T. 1989. Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.).

Related Products

Nucleic Acid Labeling

PHOTOPROBE® Biotin (SP-1000) for fast and simple biotinylation.
FastTag® Labeling System (MB-8000) for labeling with any thiol-reactive label shown in Note F.
3' EndTag™ (MB-9002) for nucleic acid labeling.

Detection of Nucleic Acids

UltraSNAP™ Chemiluminescent/Fluorescent Detection Kit (MB-6500) for biotinylated probes.
Alkaline Phosphatase Streptavidin (SA-5100) for detection of biotin.
Alkaline Phosphatase Anti-Fluorescein Antibody (MB-2100) for detection of fluorescein.
Alkaline Phosphatase Anti-Rhodamine Antibody (MB-1920) for detection of Texas Red®.
Alkaline Phosphatase Anti-DNP Antibody (MB-3100) for detection of dinitrophenyl.
DuoLuX™ Chemiluminescent/Fluorescent Substrate (SK-6605) for Alkaline Phosphatase.
BCIP/NBT Colorimetric Substrate (SK-5400) for Alkaline Phosphatase.

Immobilization of Nucleic Acids

VECTREX® Avidin D (A-2020) for irreversible binding of biotinylated nucleic acids.
VECTREX® Avidin DLA (MB-2021) for reversible binding of bioinylated nucleic acids.
VECTREX® Avidin AAL (MB-1396) for reversible binding of fucose-labeled nucleic acids.

Ancillary Products

10x Casein Solution (SP-5020) for blocking of blotting membranes.
Biotinylated DNA Molecular Weight Markers (MB-1302).
NicKit™ *p.s.o.* **(MB-1905)** probe size optimization kit.

For a complete listing and description of Vector Laboratories labeling and detection products please consult the Vector Laboratories catalog, visit our website, or contact our Technical Service Department for assistance.

This kit is designed for research use only.

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